Covalent Intermediate Trapped in KDPG Aldolase Structure at 1.95Å Resolution

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Introduction: 2-Keto-3-deoxy-6-phosphogluconate (KDPG) aldolase catalyses the reversible cleavage of KDPG to pyruvate and glyceraldehyde-3-phosphate. The enzyme is a class I aldolase whose reaction mechanism involves formation of Schiff base intermediates between Lys-133 and a keto substrate. A covalent adduct was trapped by flash freezing 2-keto-3-deoxy-6-phosphogluconate aldolase crystals soaked with 10mM pyruvate in acidic conditions at pH 4.6. Structure determination to 1.95Å resolution showed that pyruvate had undergone nucleophilic attack with Lys-133 forming a protonated carbinolamine intermediate, a functional Schiff base precursor, which was stabilized by hydrogen bonding with active site residues. Carbinolamine interaction with Glu-45 indicates general base catalysis of several rate steps. Stereospecific addition is ensured by aromatic interaction of Phe-135 with pyruvate methyl group. Arg-49 and Thr-73 specifically recognize and orient the incoming pyruvate carboxylate. In the native structure, Lys-133 donates all of its hydrogen bonds indicating presence of a ε-ammonium salt group. For Schiff base formation involving a lysine residue, a free ε-amino group, and not an ammonium salt group, is required as a nucleophile. It is proposed that Lys-133 acts as a general acid catalyst during carbinolamine formation, which upon proton transfer to the incoming pyruvate carbonyl oxygen, creates a reactive electrophile for subsequent nucleophilic attack by Lys-133.

Methods and Materials: Crystals were cryoprotected by transfer to mother liquor made up with 20 % glycerol as well as 10mM pyruvate in case of pyruvate soaked crystals. Crystal were soaked for 15 minutes and then flash frozen at -170°C. Crystals have space group $P2_12_12_1$ with unit cell dimensions a=54.95 Å, b=85.21 Å, c=133.69 Å and a trimer in the asymmetric unit cell. Unit cell dimensions (a=54.67Å, b=96.42Å, c=120.12Å) but not space group differed for the SeMet derivative with respect to native crystals and these were similar in presence or absence of pyruvate. The asymmetric unit cell contents were nevertheless consistent with a trimeric quaternary structure for the SeMet derivative. Anomalous pairs for the SeMet:pyruvate derivative were collected in a single pass using inverse-normal beam geometry at three different wavelengths. All data sets were processed independently using the programs DENZO and SCALEPACK[1].

The MAD data were scaled by the program SOLVE [2] and eleven of twelve selenium sites including the N-terminus substituted methionines were obtained for the SeMet:pyruvate derivative. Three-fold non-crystallographic symmetry (NCS) was deduced from SeMet positions. Initial phases were improved by the DM program [3] and included NCS averaging. Model building was initiated by manual fitting of a 2-keto-3-deoxy-6-phosphogluconate aldolase model (kindly provided by Professor Tulinsky, Michigan State) into the MAD density using O[4]. The KDPG aldolase trimer was then subjected to iterative rounds of model building and refinement using CNS [5] for all data extending to 2.0Å.

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